ZooKeys 1070: 13–30 (2021)
doi: 10.3897/zookeys.1070.72742
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Characterization of the complete mitochondrial genome of *Myrmus lateralis* (Heteroptera, Rhopalidae) and its implication for phylogenetic analyses

Wanqing Zhao[1], Dajun Liu[1], Qian Jia[1], Xin Wu[1], Hufang Zhang[1]

1 *Department of Biology, Xinzhou Teachers University, Xinzhou 034000, Shanxi, China*

Corresponding author: Hufang Zhang (zh_hufang@sohu.com)

Academic editor: Laurence Livermore | Received 12 August 2021 | Accepted 25 October 2021 | Published 10 November 2021

http://zoobank.org/9F874E62-5B59-408F-9810-8539A3C5E85A

Citation: Zhao W, Liu D, Jia Q, Wu X, Zhang H (2021) Characterization of the complete mitochondrial genome of Myrmus lateralis (Heteroptera, Rhopalidae) and its implication for phylogenetic analyses. ZooKeys 1070: 13–30. https://doi.org/10.3897/zookeys.1070.72742

Abstract

Mitochondrial genomes (mitogenomes) are widely used in research studies on phylogenetic relationships and evolutionary history. Here, we sequenced and analyzed the mitogenome of the scentless plant bug *Myrmus lateralis* Hsiao, 1964 (Heteroptera, Rhopalidae). The complete 17,309 bp genome encoded 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and a control region. The mitogenome revealed a high A+T content (75.8%), a positive AT-skew (0.092), and a negative GC-skew (–0.165). All 13 PCGs were found to start with ATN codons, except for *cox1*, in which TTG was the start codon. The Ka/Ks ratios of 13 PCGs were all lower than 1, indicating that purifying selection evolved in these genes. All tRNAs could be folded into the typical cloverleaf secondary structure, except for *trnS1* and *trnV*, which lack dihydrouridine arms. Phylogenetic trees were constructed and analyzed based on the PCG+rRNA from 38 mitogenomes, using maximum likelihood and Bayesian inference methods, showed that *M. lateralis* and *Chorosoma macilentum* Stål, 1858 grouped together in the tribe Chorosomatini. In addition, Coreoidea and Pyrrhocoroidea were sister groups among the superfamilies of Trichophora, and Rhopalidae was a sister group to Alydidae + Coreidae.

Keywords

Mitogenome, next-generation sequencing, phylogeny, scentless plant bugs

Copyright *Wanqing Zhao et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Mitochondria are important cytoplasmic organelles in eukaryotic cells, playing a critical role in cell metabolism, disease, apoptosis, and senescence. Mitochondrial genomes are characterized by a simple structure, stable composition, conserved arrangement, maternal inheritance, and rapid evolutionary rate (Simon et al. 2006; Cameron 2014; Tyagi et al. 2020). Therefore, mitochondrial genomes (mitogenomes) have been widely used in phylogenetic analyses and studies on population genetics (Yuan et al. 2015; Song et al. 2019; Xu et al. 2021). For most insects, the mitogenome is typically a double-stranded circular DNA molecule from 15 to 20 kb in size, including 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, as well as a long non-coding region, also known as the control or AT-rich region (Wolstenholme 1992; Boore 1999; da Fonseca et al. 2008; Yang et al. 2018). Next-generation sequencing enables large amount of sequence data to be obtained and analyzed in a more economically efficient manner. As a result of advances in this technology, it is now more feasible to obtain the complete mitogenome of large taxa; thus, molecular phylogenetic analysis has been revolutionized (Beckenbach et al. 2009; Kocher et al. 2014).

Rhopalidae, commonly known as scentless plant bugs, is a family of Pentatomomorpha insects in the superfamily Coreoidea (Schaefer and Chopra 1982; Schaefer and Panizzi 2000; Steill and Meyer 2003). The Rhopalidae includes 26 genera and 279 species distributed throughout the biogeographical areas of the world (CoreoideaSF Team 2021); many species are of critical importance as pests of pasture grass (Carroll and Loye 2012). In China, Rhopalidae includes two subfamilies, Serinethinae (containing only one genus) and Rhopalinae (containing 20 genera) (Dolling 2006). Phylogeny for the tribes of Rhopalinae has been hypothesized based on morphology, but several concurrent hypotheses still exist (Schaefer 1993; Li and Zheng 1994; Davidova-Vilimova et al. 2000; Henry 2019). Although there are several published studies examining the relationships among infrafamilial levels of the Coreoidea, a consensus regarding the relationships among different families and subfamily lineages has not been reached (Souza et al. 2009; Ghahari et al. 2012; Forthman et al. 2019). In addition, some studies support Aradoidea as sister group of Trichophora (which includes Pentatomoidea, Lygaeoidea, Pyrrhocoroidea and Coreoidea), but the relationship within Trichophora is still under debate (Li et al. 2005; Xie et al. 2005; Linnavuori 2007; Tian et al. 2011; Weirauch and Schuh 2011).

Myrmus lateralis Hsiao, 1964 is an endemic species of China (Beijing, Hebei, eastern Inner Mongolia), Korea, Mongolia, and Russia (Far East) (Liu et al. 1994; Aukema and Rieger 2006; CoreoideaSF Team 2021). Adults are present in both forests and grasslands during July and August (Nonnaizab 1986). To date, only four complete mitogenomes from the Rhopalidae have been published in GenBank, and all of them belong to Rhopalinae (Table 1). According to the latest classification, five tribes are classified in the Rhopalidae (CoreoideaSF Team 2021). The tribe Chorosomatini Fieber, 1860 contains five genera (*Agraphopus* Stål, 1872; *Chorosoma* Curtis, 1830; *Ithamar* Kirkaldy, 1902; *Leptoceraea* Jakovlev, 1873; *Myrmus* Hahn, 1832 and *Xenogenus* Berg, 1883), of which only *Myrmus* and *Chorosoma* are distributed in China.

Table 1. List of sequences used to reconstruct the phylogenetic tree.

	Family	Species	Accession number
Outgroup	Nabidae	*Himacerus nodipes*	JF927832
Ingroup	Aradidae	*Aradacanthia heissi*	HQ441233
		Aradus compar	NC_030362
		Aneurus similis	NC_030360
	Cydnidae	*Macroscytus gibbulus*	NC_012457
		Adrisa magna	NC_042429
		Scoparipes salvazai	MF614955
	Pentatomidae	*Eurydema dominulus*	NC_044762
		Palomena viridissima	NC_050166
		Eysarcoris guttigerus	MN831205
		Arma custos	MT535604
		Pentatoma semiannulata	MT985377
	Colobathristidae	*Phaenacantha marcida*	EU427342
	Rhyparochromidae	*Panaorus albomaculatus*	NC_031364
	Malcidae	*Malcus inconspicuus*	EU427339
	Lygaeidae	*Kleidocerys resedae*	KJ584365
	Pyrrhocoridae	*Dindymus rubiginosus*	NC_042439
		Euscopus rufipes	NC_042436
		Melamphaus faber	NC_042435
		Dysdercus decussatus	NC_042438
		Antilochus coquebertii	NC_042441
	Rhopalidae	*Stictopleurus subviridis*	EU826088
		Myrmus lateralis	**MN412595**
		Chorosoma macilentum	MN412594
		Aeschyntelus notatus	EU427333
		Corizus sp.	KM983397
	Alydidae	*Riptortus pedestris*	EU427344
		Neomegalotomus parvus	MG253274
	Coreidae	*Hydaropsis longirostris*	EU427337
		Clavigralla tomentosicollis	KY274846
		Acanthocoris sp.	MF497707
		Enoplops potanini	MF497720
		Leptoglossus membranaceus	MF497724
		Anoplocnemis curvipes	KY906099
		Mictis tenebrosa	MF497729
		Pseudomictis brevicornis	MF497732
		Molipteryx lunata	MF497721
		Notopteryx soror	KX505857

In this study, we sequenced and annotated the complete mitogenome of *M. lateralis*. We examined the genomic features, nucleotide composition, codon usage, RNA secondary structure, evolutionary pattern of 13 PCGs and characteristics of the control region. Finally, we evaluated the phylogenetic relationship of the mitochondrial sequence data at different taxonomic levels.

Material and methods

Sampling, DNA extraction and sequencing

Adult specimens of *Myrmus lateralis* were collected from Jincheng City, Shanxi Province, China on 20 July 2014. Specimens were preserved in 100% ethanol and stored at –20 °C. The genomic DNA was extracted from leg muscles using a ONE-4-ALL

Genomic DNA Mini-Prep Kit (BS88504; Sangon, Shanghai, China) according to the manufacturer's protocols. The mitogenome was sequenced using the whole-genome shotgun method on an Illumina Miseq platform (Personalbio, Shanghai, China). After filtering low-quality and adapter contaminated reads, A5-miseq version 20150522 (Coil et al. 2015) was used for contig assembly.

Gene annotation and sequence analysis

Sequence annotation was performed using Geneious 10.1.3 (Kearse et al. 2012) and the MITOS web server (Bernt et al. 2013). Annotations of 13 protein-coding regions were edited manually by predicting open reading frames using the invertebrate mitochondrial code. The secondary structures of the tRNA genes were predicted with tRNAscan-SE web server (http://lowelab.ucsc.edu/tRNAscan-SE/) (Tamura et al. 2013). The identification of rRNA genes was performed based on their putative secondary structures and by comparing nucleotide sequences with those of previously reported mitogenomes. The control region was identified through the boundary of neighboring genes. The number of tandem repeats of control region was investigated with Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.html) (Benson 1999) and the presence of stem-loop was predicted by Mfold Web Server (http://mfold.rna.albany.edu) (Zuker 2003). The annotated mitogenome sequence of *Myrmus lateralis* has been submitted to GenBank under the accession number MN412595.

The base compositions, codon usages, and relative synonymous codon usage (RSCU) values were calculated by MEGA 6.0. AT and GC skew were calculated by the following formulae: AT skew = (A–T)/(A+T) and GC skew=(G–C)/(G+C). To analyse the evolutionary patterns of the 13 PCGs, the rates of nonsynonymous substitution (Ka), the rates of synonymous substitution (Ks), and the ratio of Ka/Ks for each gene were calculated by the software DnaSP 5.0 (Librado and Rozas 2009).

Phylogenetic analyses

In addition to the mitogenome newly sequenced here, 37 other mitogenomes were taken from Genbank for the phylogenetic analyses (Table 1). The nucleic acids of 13 PCGs and two rRNAs were extracted by Geneious 10.1.3 and aligned with the Muscle algorithm in MEGA 6.0. Finally, the individual alignments were concatenated to make the datasets of PCG+rRNA (nucleotide alignment including 13 protein-coding genes and two rRNA genes) with SequenceMatrix (Vaidya et al. 2011).

The dataset was used to reconstruct phylogenetic trees under Bayesian inference (BI) and maximum likelihood (ML) using MrBayes 3.2.6 (Ronquist et al. 2012) and RaxML 8.0.2 (Stamatakis 2015) respectively. BI analysis was performed using a TVM+I+G model selected by jModeltest (Darriba et al. 2012). Two independent runs of four Markov Chains Monte Carlo (MCMC) chains in parallel for 5,000,000 generations were applied and sampled every 1,000 generations. Tracer 1.7 (Rambaut et al. 2018) was used to analyze the trace files from the Bayesian MCMC runs. Stationarity was

considered to be reached when ESS (estimated sample size) value above 100 as MrBayes suggested. The first 25% of sampled trees were discarded as burn-in, and the remaining trees were used to calculate a 50% majority rule consensus tree. The ML tree was constructed using the GTR+I+G model, and nodal support values were evaluated through an ultrafast bootstrap approach, with 10,000 replicates.

Results

Genome organization

The 17,309 bp mitochondrial genome of *Myrmus lateralis* was determined to be a typical circular nucleotide molecule, consisting of 13 PCGs, 22 tRNA genes, two rRNA genes, and a putative control region (Table 2). For the gene arrangement, 23 genes (nine PCGs and 14 tRNAs) were found on the majority strand and 14 genes (four PCGs, eight tRNAs, and two rRNAs) were found on the minority strand. In addition, the mitogenome contains 10 gene overlaps totaling 37 bp and ranging from 1 to 8 bp; the longest overlap is being found between *trnW* and *trnC*. Thirteen intergenic spacers equal to 77 bp were observed, ranging from 1 to 18 bp, the longest being found between *trnS2* and *nad1*.

Nucleotide composition and codon usage

The mitochondrial genome of *M. lateralis* was strongly biased toward A+T (75.8%) in nucleotide composition, with 75.5%, 75.7%, 71.3%, and 74.8% A+T content in PCGs, rRNAs, tRNAs, and control regions, respectively. The nucleotide composition and skewness of the mitogenome is shown in Table 3. The three codon positions of PCGs possessed different A+T content; the third site had the highest value (80.1%), whereas the second site had the lowest (74.3%). The mitogenome had more A and G content than T and C, with a positive AT-skew (0.092) and a negative GC-skew (–0.165). The AT-skew of PCGs and three codon positions were all negative, –0.018 in PCGs, –0.214 in the first, –0.050 in the second, and –0.099 in the third codon positions. The GC-skew of PCGs (0.033) and the second codon position (0.127) was slightly and moderately positive, respectfully, while the first and third codon positions were both negative (–0.018 and –0.020, respectively). AT-skew values for the tRNA and rRNA genes were both negative (–0.057 and –0.001, respectively), while the GC-skew was obviously positive for tRNAs (0.292) and slightly positive for rRNAs (0.019).

Relative synonymous codon usage (RSCU) values of the 13 PCGs were calculated based on 3633 codons (Fig. 1). UUU (F, Phe), UAU (Y, Tyr), UUA (L, Leu2), and AUU (I, Ile) were the most frequently used codons, accounting for 28.24% of all codons. All synonymous codons ending with A or U were more frequent than those ending with C or G, except in three cases: CGG (RSCU = 0.97) was used more than CGU (RSCU = 0.55) for Arg, AGG (RSCU=1.03) was used more than AGA (RSCU = 0.99) for Ser1, and GGG (RSCU=1.27) was used more than GGU (RSCU = 1.12) for Gly.

Table 2. Organization of the mitochondrial genome of *Myrmus lateralis*.

Gene	Strand	Size(bp)	Position		Intergenicnucleotides(IGN)	Antico-don	Codons	
			Start	Stop			Start	Stop
trnI	J	66	1	66	0	GAU	—	—
trnQ	N	69	64	132	2	UUG	—	—
trnM	J	69	137	205	4	CAU	—	—
nad2	J	985	207	1191	1	—	ATG	T
trnW	J	63	1204	1266	12	UCA	—	—
trnC	N	62	1259	1320	-8	GCA	—	—
trnY	N	64	1321	1384	0	GUA	—	—
cox1	J	1539	1387	2925	2	—	TTG	TAA
trnL2^{UUR}	J	66	2921	2986	-5	UAA	—	—
cox2	J	676	2987	3662	0	—	ATT	T
trnK	J	72	3663	3734	0	CUU	—	—
trnD	J	65	3738	3802	3	GUC	—	—
atp8	J	162	3803	3964	0	—	ATA	TAA
atp6	J	669	3958	4626	-7	—	ATG	TAA
cox3	J	790	4626	5415	-1	—	ATG	T
trnG	J	63	5413	5475	-3	UCC	—	—
nad3	J	349	5476	5824	0	—	ATA	T
trnA	J	63	5825	5887	9	UGC	—	—
trnR	J	64	5891	5954	3	UCG	—	—
trnN	J	66	5954	6019	-1	GUU	—	—
trnS1^{AGN}	J	69	6023	6091	3	GCU	—	—
trnE	J	64	6091	6154	-1	UUC	—	—
trnF	N	66	6154	6219	-1	GAA	—	—
nad5	N	1702	6220	7921	0	—	ATA	T
trnH	N	63	7931	7993	9	GUG	—	—
nad4	N	1317	7994	9310	0	—	ATG	TAA
nad4L	N	288	9304	9591	-7	—	ATT	TAA
trnT	J	62	9594	9655	3	UGU	—	—
trnP	N	64	9656	9719	0	UGG	—	—
nad6	J	483	9728	10210	8	—	ATA	TAA
cytB	J	1137	10210	11346	-1	—	ATG	TAG
trnS2^{UCN}	J	69	11345	11413	-2	UGA	—	—
nad1	N	927	11432	12358	18	—	ATT	TAA
trnL1^{CUN}	N	67	12359	12425	0	UAG	—	—
rrnL	N	1261	12426	13686	0	—	—	—
trnV	N	69	13687	13755	0	UAC	—	—
rrnS	N	967	13756	14722	0	—	—	—
Control region		2587	14723	17309	0	—	—	—

Note: J refers to major strand; N refers to minus strand

Table 3. Nucleotide composition and skewness of the *Myrmus lateralis* mitochondrial genome.

Feature	T%	C%	A%	G%	A+T%	AT-skew	GC-skew
Whole genome	34.4	14.1	41.4	10.1	75.8	0.092	-0.165
PCGs	42.2	11.8	33.3	12.6	75.5	-0.118	0.033
PCGs-1st	44	14.1	28.5	13.6	72.5	-0.214	-0.018
PCGs-2nd	39	11	35.3	14.2	74.3	-0.05	0.127
PCGs-3rd	44	10.4	36.1	10	80.1	-0.099	-0.02
tRNAs	40	8.6	35.7	15.7	75.7	-0.057	0.292
rRNAs	35.7	14.1	35.6	14.7	71.3	-0.001	0.019
Control region	35	15.9	39.8	9.4	74.8	0.064	-0.257

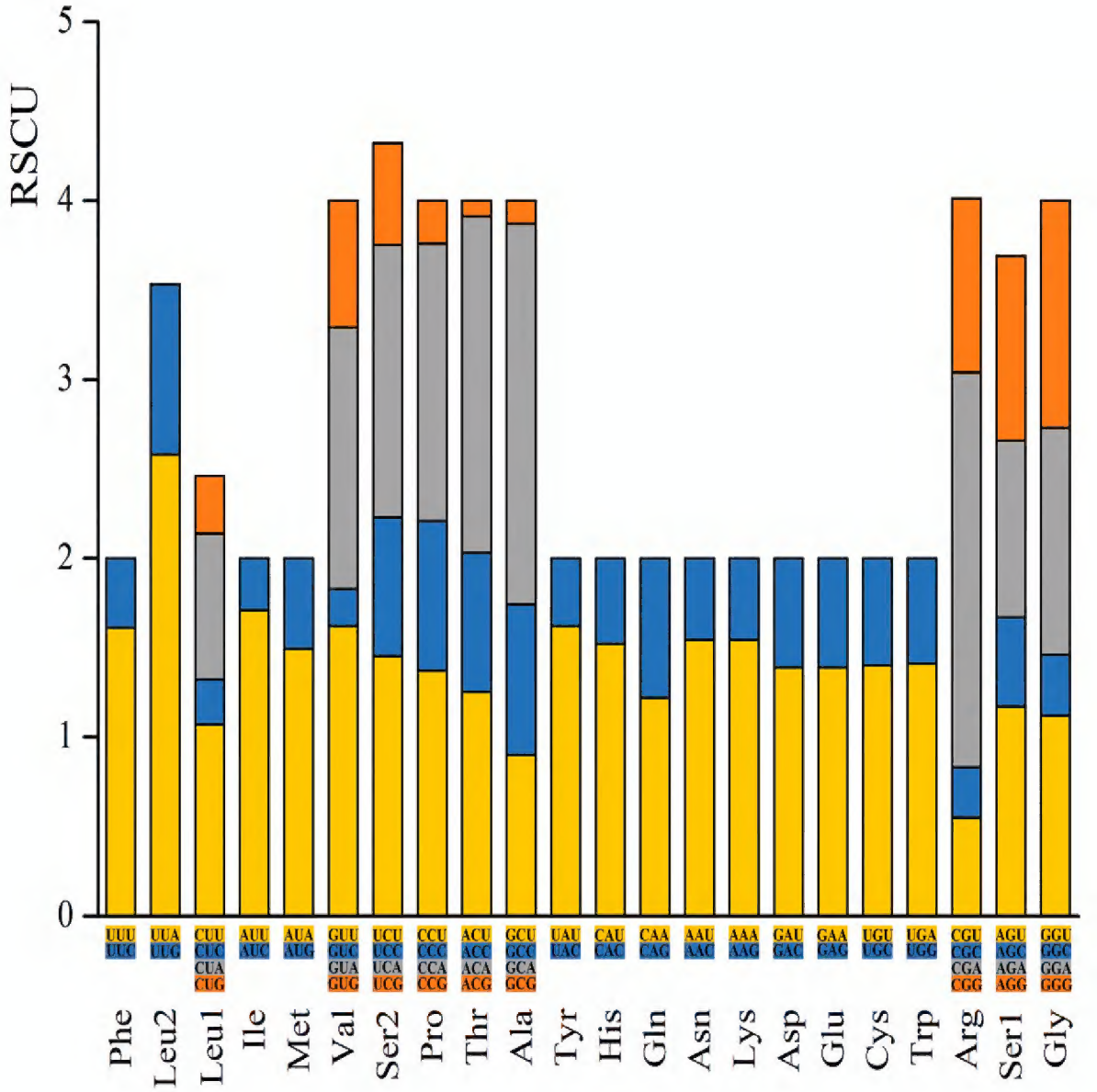

Figure 1. The relative synonymous codon usage (RSCU) in the *Myrmus lateralis* mitogenome.

Protein coding genes

In the *M. lateralis* mitogenome, nine PCGs were located on the J-strand (majority strand) and four PCGs were located on the N-strand (minority strand). The PCGs had a total length of 11,024 bp that accounted for 63.69% of the complete mitogenome. Among the mitochondrial proteins, Leu (16.67%), Phe (11.02%), and Tyr (9.58%) were the most frequent amino acids.

Most PCGs started with a typical ATN codon; five started with ATG (*nad2, atp6, cox3, nad4*, and *cytb*), four with ATA (*atp8, nad3, nad5*, and *nad6*), and three with ATT (*cox2, nad4L, and nad1*). The only unusual initiation codon was TTG in *cox1*. Among the 13 PCGs, seven genes ended with the complete stop codon TAA (*cox1, atp8, atp6, nad1, nad4*, and *nad4L*) or TAG (*cytb*), whereas five genes ended with the partial termination codon T (*nad2, cox2, cox3, nad3*, and *nad5*).

The evolutionary patterns of the 13 PCGs were analyzed and shown in Figure 2. The highest values for both Ka and Ks were observed for *atp8 and cytb*. *atp8* exhibited the highest Ka/Ks value, whereas *cox1* exhibited the lowest. The Ka/Ks values for all 13 PCGs were below 0.29.

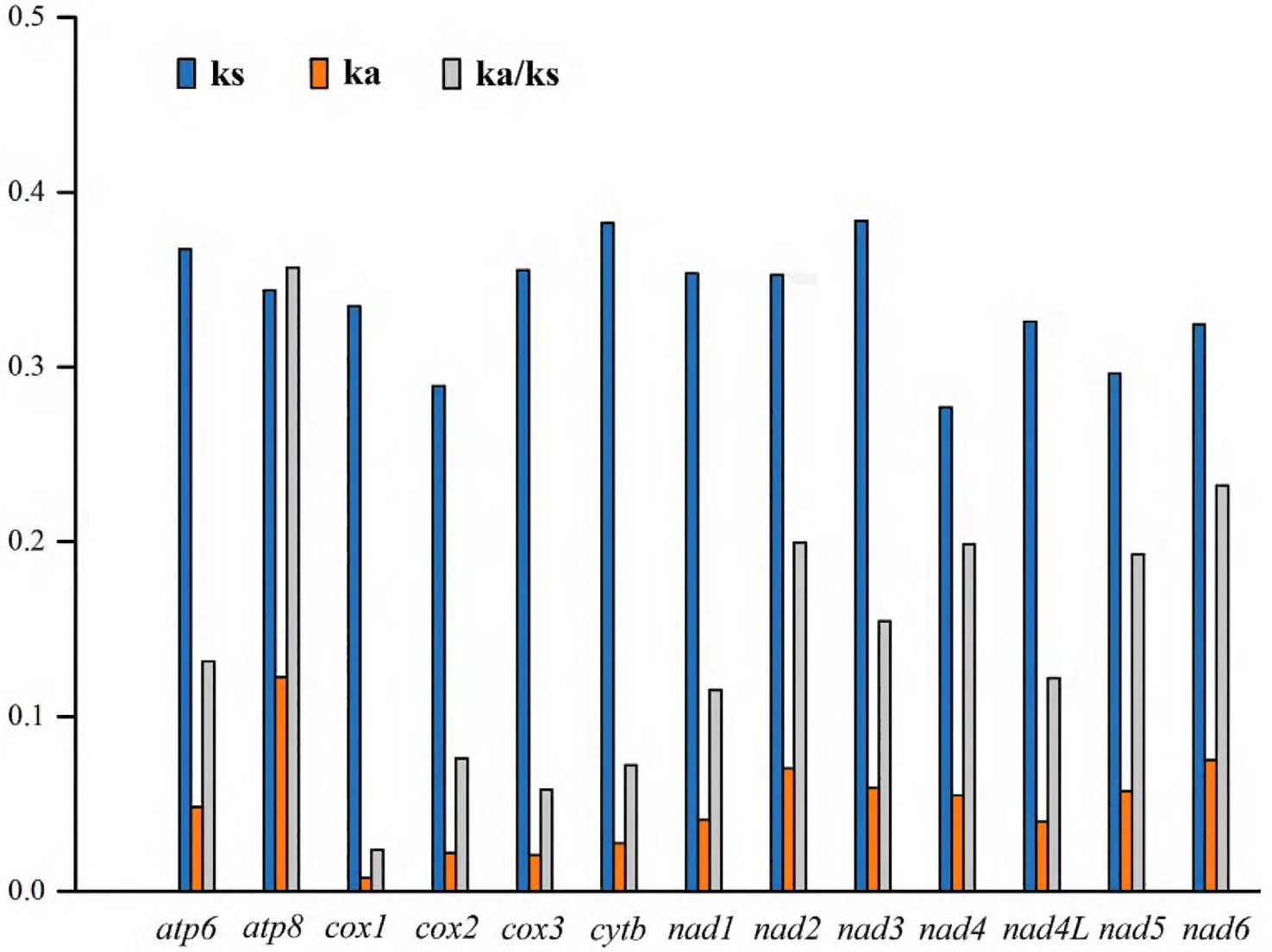

Figure 2. The rates of nonsynonymous substitution (Ka), the rates of synonymous substitution (Ks), and the ratio of Ka/Ks for each PCGs of *Myrmus lateralis* mitogenome

Transfer RNAs and ribosomal RNAs

A total of 22 tRNA genes were encoded by the *M. lateralis* mitogenome, ranging from 62 bp (*trnC* and *trnT*) to 72 bp (*trnK*) in length (Table 2). Eight tRNA genes (*trnQ, trnC, trnY, trnF, trnH, trnP, trnL1*$^{(CUN)}$ and *trnV*) were encoded on the N-strand; the remaining 14 genes were encoded on the J-strand. The 22 tRNA genes had a total length of 1,445 bp, accounting for 8.34% of the complete mitogenome. The predicted secondary structures are shown in Figure 3. All tRNA genes could be folded into typical cloverleaf secondary structures, except for *trnS1* and *trnV*, in which the necessary dihydrouridine (DHU) arms were replaced with a simple loop. A total of 16 wobbled G-U pairs were found (six in acceptor stems, eight in DHU stems, one anticodon stems, and one in TΨC stems) that formed weak bonds in the tRNAs.

The large 1,261 bp *rrnL* gene was located between *trnL1* $^{(CUN)}$ and *trnV*, and the small 967 bp *rrnS* gene was located between *trnV* and the control region. The secondary structures of both the *rrnL* and *rrnS* genes are shown in Figures 4, 5. The secondary structure of the *rrnL* gene contained five domains (I, II, IV, V, VI); domain III was absent, whereas the *rrnS* gene consisted of three domains (I, II, III). Both rRNAs included many mismatched base pairs, most of which were G-U.

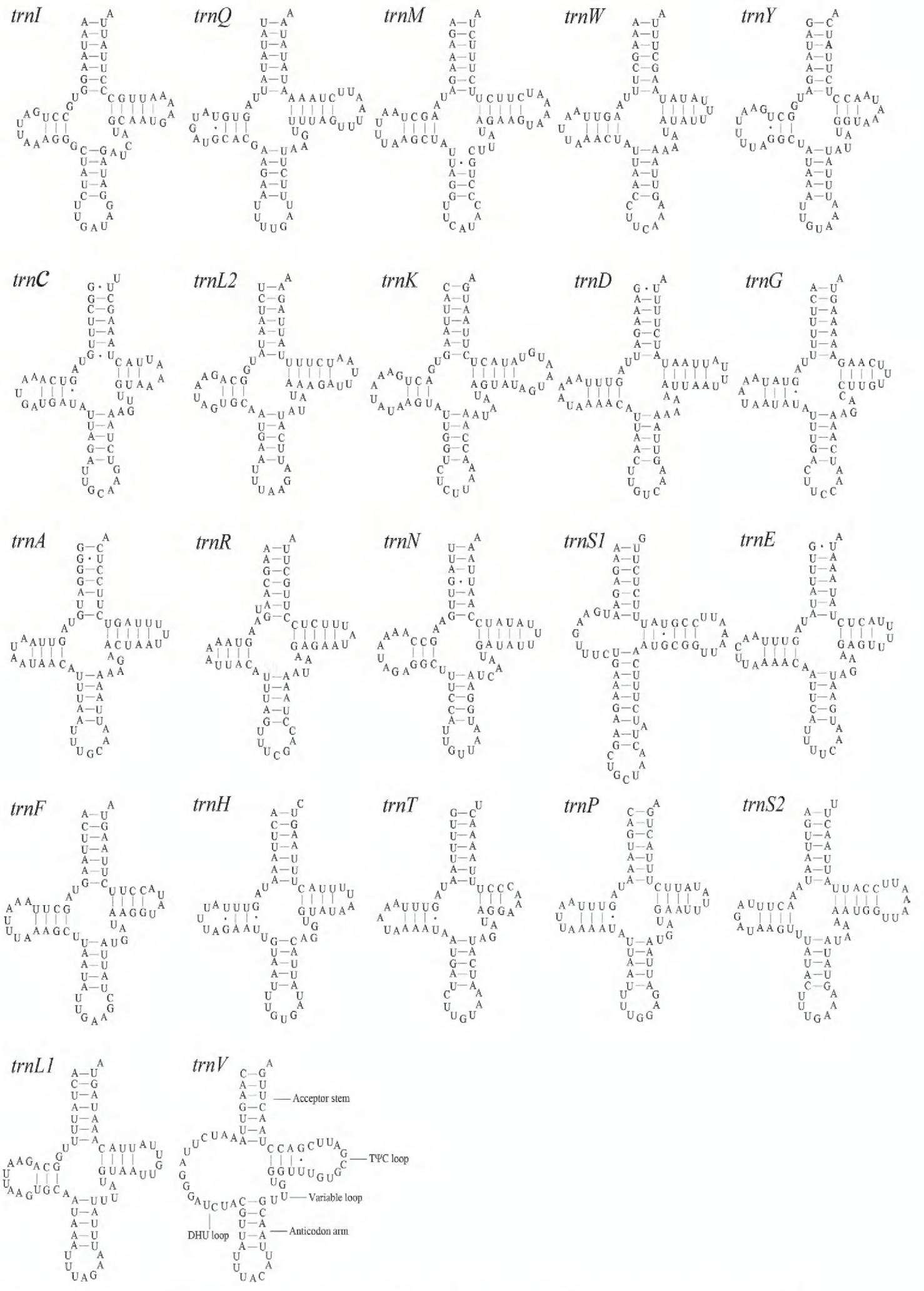

Figure 3. Predicted secondary structure of tRNA genes in the *Myrmus lateralis* mitogenome.

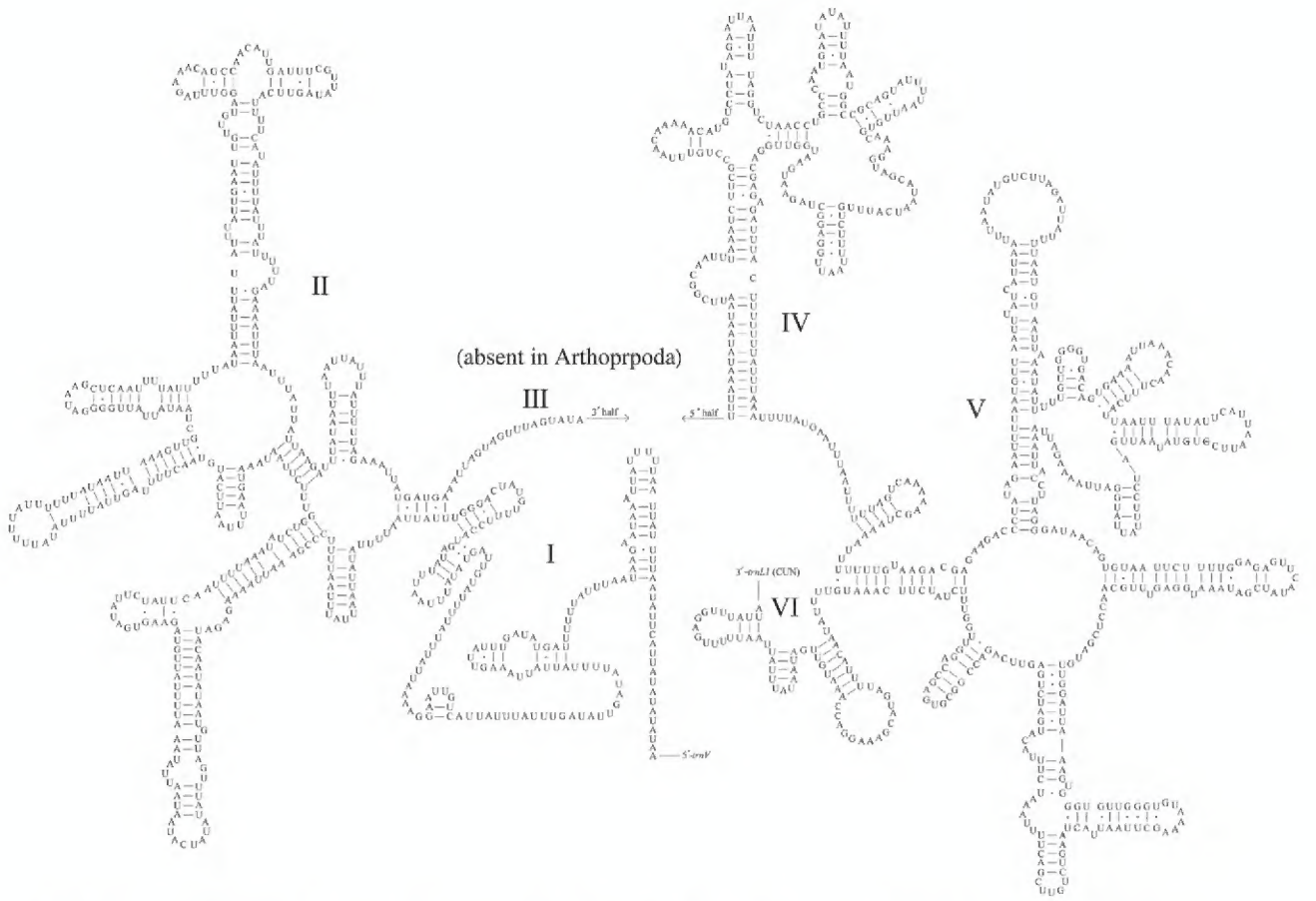

Figure 4. Predicted secondary structure of the *rrnL* in the *Myrmus lateralis* mitogenome.

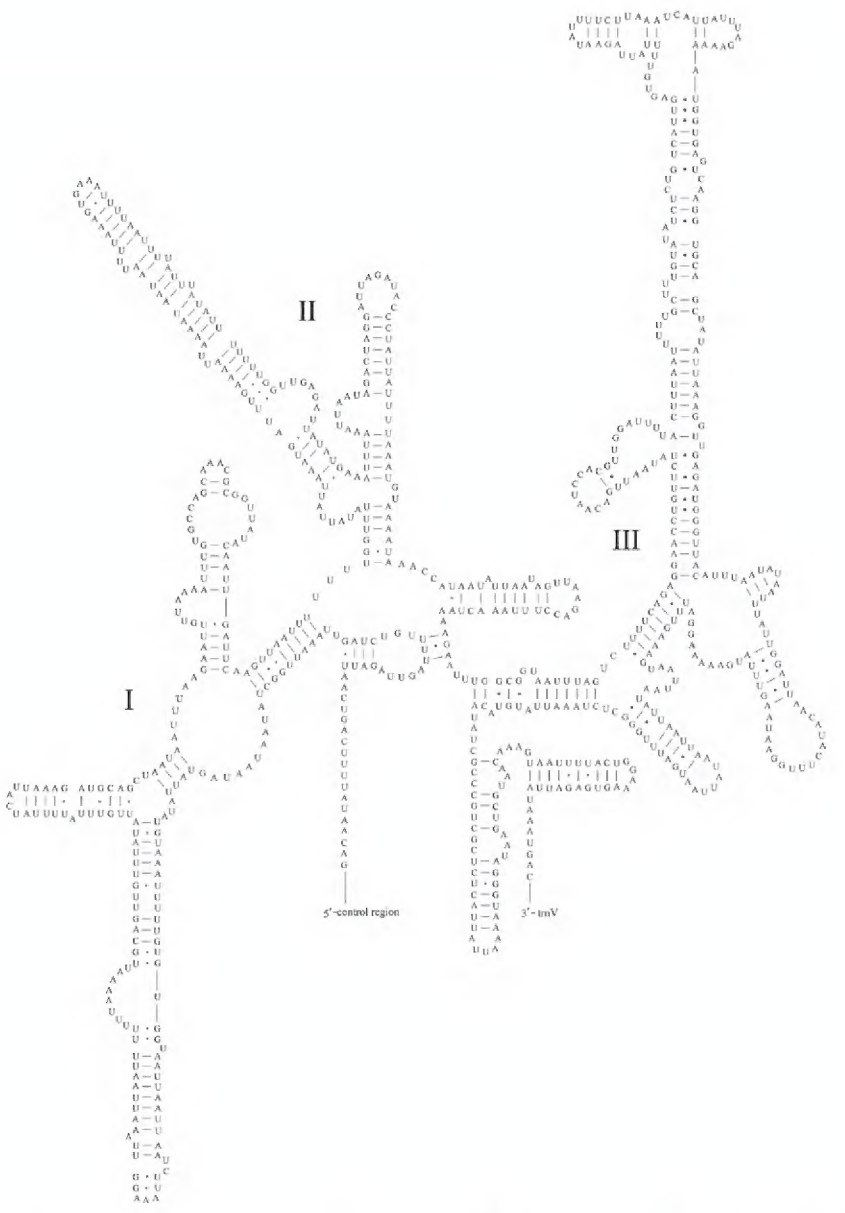

Figure 5. Predicted secondary structure of the *rrnS* in the *Myrmus lateralis* mitogenome.

Control region

The 2587 bp mitochondrial control region of *M. lateralis* was located between the *rrnS* gene and *trnI*. The A+T content (74.76%) was higher than the G+C content (25.24%), with a positive AT-skew and a negative GC-skew. In the control region, tandem repeat sequences, polyT stretch, polyA stretch, stem-loop structure, tandem repeats, and $G(A)_n$ motif were commonly found. Based on these features, we identified the following important elements: a 13 bp polyT stretch, a $G(A)_9T$ sequence, an ATAGA motif, and a 9 bp polyA stretch. In addition, a stem-loop structure was observed at the end of the control region (Fig. 6). Although no longer tandem repeat sequences were identified, stretches of $T(A)_4$ occurred many times.

Phylogenetic analyses

We conducted phylogenetic analyses based on the nucleotide sequences of the PCG+rRNA from eleven families within five superfamilies; one species from the Nabidae (damsel bugs) was used as an outgroup. The dataset contained 14,128 nucleotide sites from these 38 taxa. The BI and ML analyses generated identical phylogenetic results with most posterior probabilities (PP) of one and bootstrap pseudoreplicates (BP) of 100 (Fig. 7). Pentatomoidea, Lygaeoidea, Pyrrhocoroidea, and Coreoidea, the trichophorans, were all monophyletic and highly supported in both analyses (PP > 0.85 and BP > 92). A sister relationship between Coreoidea and Pyrrhocoroidea was recovered, and Pentatomoidea formed a sister group with the other superfamilies of the remaining Trichophora. Both analyses provided robust support (PP > 0.95 and BP > 85) for three families within Coreoidea: Alydidae was closer to Coreidae than Rhopalidae. Sub-

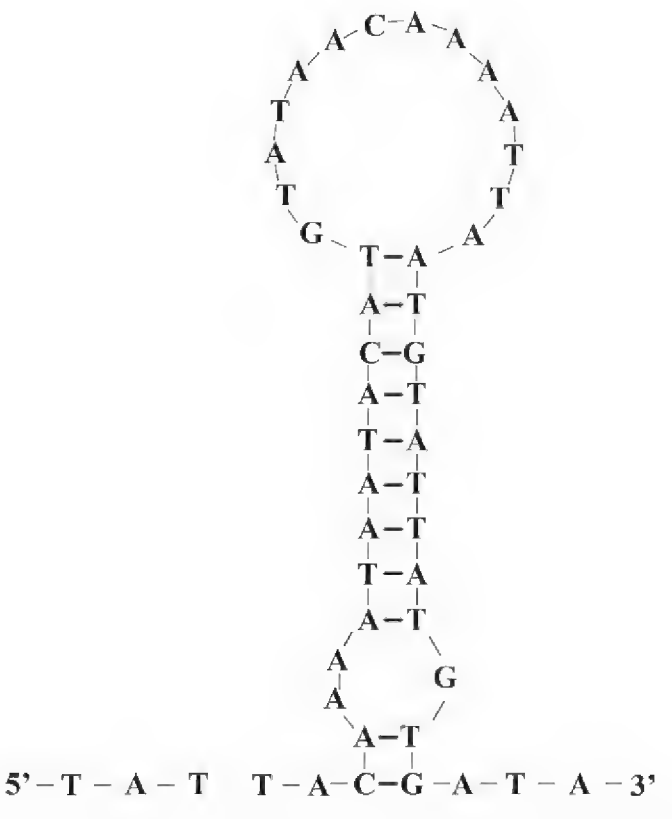

Figure 6. The stem-loop of control region in the *Myrmus lateralis* mitogenome.

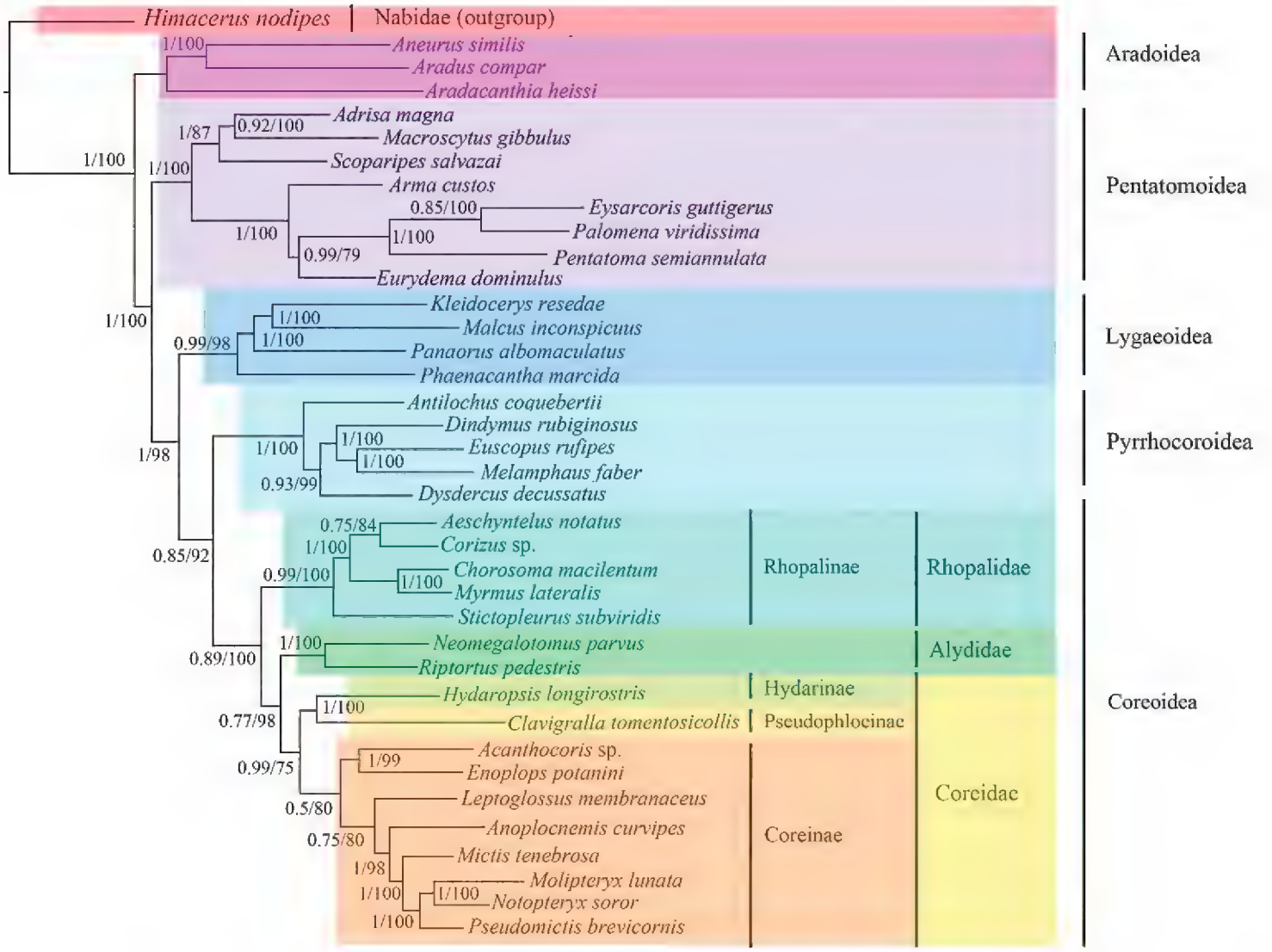

Figure 7. The phylogenetic relationships of PCG+rRNA using BI and ML methods. Numbers above each node indicate Bayesian posterior probabilities values and ML bootstrap values.

families of Coreidae were also supported with an internal relationship of ((Hydarinae + Pseudophloeinae) + Coreinae) (PP > 0.77 and BP > 75). Among Rhopalinae species, *Stictopleurus subviridis* Hsiao, 1977 was identified as sister to a group of other Rhopalinae species (PP = 0.99 and BP = 100). *M. lateralis* was close to *Chorosoma macilentum* Stål, 1858 (PP = 1 and BP = 100) and they both belonged to the tribe Chorosomatini.

Discussion

In this study, we sequenced the mitogenome of *Myrmus lateralis* and built the molecular phylogenetic relationships with 37 other heteropteran taxa. The mitogenome was found to be 17,309 bp long, with 37 genes arranged consistent with other species of Hemiptera (Song et al. 2012; Li et al. 2017; Zhao et al. 2021). The control region represented the largest reported non-coding region for insect mitogenomes owing to its various motifs and tandem repeats (Cook et al. 1999; Li et al. 2015). Although the 2587 bp long control region possessed several elements, such as a polyT stretch, a $G(A)_nT$ sequence, an ATAGA motif, a stem-loop, and a polyA stretch, tandem repeats were not observed.

The present study analyzed the initiation and termination codons of the 13 PCGs. The majority of PCGs used common start codons (ATN), except for *cox1*, which was initiated with TTG, a frequently used start codon in heteropteran mitogenomes (Quek et al. 2021; Zhao et al. 2021). The partial termination codon T was found in *nad2, cox2, cox3, nad3* and *nad5*. Some researchers have proposed that the incomplete termination codon could be completed via post-transcriptional polyadenylation (Ojala et al. 1981). Among the synonymous codons, codons ending with an A or U were more frequent than those ending in a G or C and were also observed in other heteropteran species (Zhao et al. 2019; Li et al. 2021). In this study, the lowest evolutionary rate was observed in *cox1*, indicating that this gene can be effectively used as a DNA barcoding marker. The Ka/Ks values for all 13 PCGs were far less than one, indicating that all of the PCGs evolved under purifying selection, thus, they can be used to investigate phylogenetic relationships.

Although many studies have reconstructed phylogenetic relationships among members of Pentatomomorpha, the relationships of different levels are still ambiguous (Li et al. 2005; Liu et al. 2019; Souza-Firmino et al. 2020; Kaur and Singh 2021). The results from previous research were congruent with the result that Trichophora includes four superfamilies of Pentatomomorpha except Aradoidea, and with the monophyly of four superfamilies of Trichophora (Henry 1997; Hua et al. 2008; Yuan et al. 2015; Song et al. 2019). Hua et al. (2008) and Liu et al. (2019) supported Pentatomoidea as the basal group of Trichophora and Coreoidea as a sister group to Lygaeoidea based on mitochondrial genomes. The superfamilies Coreoidea and Pyrrhocoroidea were supported as sister taxa based on cladistic analysis (Henry 1997), as well as 37 mitochondrial genes analysis (Yuan et al. 2015). Whereas our mitogenomic data showed that Coreoidea and Pyrrhocoroidea were sister groups based on ML and BI analyses using the PCG+rRNA dataset. This result from our study was consistent with that from the study of Song et al. (2019) based on relationship analysis of Pentatomomorpha PCGs.

At the family level, Rhopalidae was found to be a sister group to Alydidae + Coreidae. This relationship, based on mitogenomes, was consistent with that based on cladistic analysis by Li (1996) and Henry (1997). Despite the availability of numerous species, the classification patterns for the family Coreidae were weak in terms of both morphology and molecular data (Bressa et al. 2008; Forthman et al. 2019; Froeschner 2019). In this study, Hydarinae and Pseudophloeinae grouped the sister cluster. However, due to the limited samples, the relationships among Coreidae needs more studies in the future.

Previous studies on the classification of Rhopalinae came to a variety of conclusions and the positions of some species were not clear (Schaefer and Chopra 1982; Liu 1994; Aukema and Rieger 2006). The hypothesis that the genus *Stictopleurus* belongs to the Rhopalini tribe was corroborated based on morphological studies by Aukema and Rieger (2006). However, it did not group together with other Rhopalini species in this study. The monophyly of Rhopalini should be explored in the future studies with additional specimens.

Conclusions

The complete mitogenome of the scentless plant bug *Myrmus lateralis* was sequenced using next-generation sequencing technologies, providing the fifth mitogenome sequence from approximately 230 species of Rhopalidae. The nucleotide composition, codon usage, RNA structures, and protein-coding genes evolution were analyzed in our paper. The mitogenome of *M. lateralis* revealed the phylogenetic position of *Myrmus*. However, more mitogenomes should be sequenced to investigate the mitogenomic evolution and phylogenetic relationships of Rhopalidae.

Acknowledgments

This project was supported by the National Science Foundation Project of China (no. 31501876, 31872272) and supported by Scientific Research Project of Xinzhou Teachers University (2018KY04).

Reference

Dolling WR (2006) Family Rhopolidae Amyot & Serville, 1843. In: Catalog of the Heteroptera of the Palaearctic Region. The Netherlands Entomological Society, Amsterdam, 8–27.

Beckenbach AT, Stewart JB (2009) Insect mitochondrial genomics 3: the complete mitochondrial genome sequences of representatives from two neuropteroid orders: a dobsonfly (order Megaloptera) and a giant lacewing and an owlfly (order Neuroptera). Genome 52(1): 31–38. https://doi.org/10.1139/G08-098

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Research 27(2): 573–580. https://doi.org/10.1093/nar/27.2.573

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF (2013) MITOS: improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69(2): 313–319. https://doi.org/10.1016/j.ympev.2012.08.023

Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Research 27(8): 1767–1780. https://doi.org/10.1093/nar/27.8.1767

Bressa MJ, Franco MJ, Toscani MA, Papeschi AG (2008) Heterochromatin heteromorphism in *Holhymenia rubiginosa* (Heteroptera: Coreidae). European Journal of Entomology 105(1): 65–72. https://doi.org/10.14411/eje.2008.009

Cameron SL (2014) Insect mitochondrial genomics: implications for evolution and phylogeny. Annual Review of Entomology 59: 95–117. https://doi.org/10.1146/annurev-ento-011613-162007

Carroll SP, Loye JE (2012) Soapberry bug (Hemiptera: Rhopalidae: Serinethinae) native and introduced host plants: biogeographic background of anthropogenic evolution. Annals of the Entomological Society of America 105(5): 671-684. https://doi.org/10.1603/AN11173

Coil D, Jospin G, Darling AE (2014) A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31(4): 587–589. https://doi.org/10.1093/bioinformatics/btu661

Cook CE, Wang Y, Sensabaugh G (1999) A mitochondrial control region and cytochromebphylogeny of sika deer (*Cervus nippon*) and report of tandem repeats in the control region. Molecular Phylogenetics and Evolution 12(1): 47–56. https://doi.org/10.1006/mpev.1998.0593

CoreoideaSF Team (2021) Coreoidea species file online. Version 5.0/5.0. http://Coreoidea.SpeciesFile.org. [Accessed on: 2021-10-26]

da Fonseca RR, Johnson WE, O'Brien SJ, Ramos MJ, Antunes A (2008) The adaptive evolution of the mammalian mitochondrial genome. BMC Genomics 9(1): 1–22. https://doi.org/10.1186/1471-2164-9-119

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature methods 9(8): 772–772. https://doi.org/10.1038/nmeth.2109

Davidova-Vilimova J, Nejedla M, Schaefer CW (2000) Dorso-abdominal scent glands and metathoracic evaporatoria in adults of central European Rhopalidae (Hemiptera: Heteroptera), with a discussion of phylogeny and higher systematics. European Journal of Entomology 97(2): 213–222. https://doi.org/10.14411/eje.2000.039

Forthman M, Miller CW, Kimball RT (2019) Phylogenomic analysis suggests Coreidae and Alydidae (Hemiptera: Heteroptera) are not monophyletic. Zoologica Scripta 48(4): 520–534. https://doi.org/10.1111/zsc.12353

Froeschner RC (1988) Family Coreidae Leach, 1815: the coreid bugs. In: Catalog of the Heteroptera, or true bugs, of Canada and the continental United States. CRC Press, Boca Raton, 69–92. https://doi.org/10.1201/9781351070447-9

Ghahari H, Moulet P, Linnavuori R, Ostovan H (2012) An annotated catalog of the Iranian Coreidae, Rhopalidae, and Stenocephalidae (Hemiptera: Heteroptera: Pentatomomorpha: Coreoidea). Zootaxa 3519(1): 1–31. https://doi.org/10.11646/zootaxa.3519.1.1

Henry TJ (1997) Phylogenetic analysis of family groups within the infraorder Pentatomomorpha (Hemiptera: Heteroptera), with emphasis on the Lygaeoidea. Annals of the Entomological Society of America 90(3): 275–301. https://doi.org/10.1093/aesa/90.3.275

Henry TJ (1988) Family Rhopalidae Amyot and Serville, 1843 (= Corizidae Douglas and Scott, 1865): the scentless plant bugs. In: Catalog of the Heteroptera, or True Bugs, of Canada and the Continental United States. CRC Press, Boca Raton, 652–664. https://doi.org/10.1201/9781351070447-37

Hua J, Li M, Dong P, Cui Y, Xie Q, Bu W (2008) Comparative and phylogenomic studies on the mitochondrial genomes of Pentatomomorpha (Insecta: Hemiptera: Heteroptera). BMC genomics 9(1): 1–15. https://doi.org/10.1186/1471-2164-9-610

Kaur R, Singh D (2021) Cytochrome b sequence divergence and phylogenetic relationships among different species of family Pentatomidae (Hemiptera: Heteroptera). International Journal of Tropical Insect Science 41(2): 1177–1183. https://doi.org/10.1007/s42690-020-00303-8

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kocher A, Kamilari M, Lhuillier E, Coissac E, Péneau J, Chave J, Murienne J (2014) Shotgun assembly of the assassin bug Brontostoma colossus mitochondrial genome (Heteroptera, Reduviidae). Gene 552(1): 184–194. https://doi.org/10.1016/j.gene.2014.09.033

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25(11): 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Linnavuori RE (2007) Studies on the Piesmatidae, Berytidae, Pyrrhocoridae, Stenocephalidae, Coreidae, Rhopalidae, Alydidae, Cydnidae, and Plataspidae (Heteroptera) of Gilan and the adjacent provinces in northern Iran. Acta Entomologica Musei Nationalis Pragae 47: 77–91. https://www.aemnp.eu/data/article-1138/1119-47_0_77.pdf

Li H, Leavengood JM, Chapman EG, Burkhardt D, Song F, Jiang P, Liu J, Zhou X, Cai W (2017) Mitochondrial phylogenomics of Hemiptera reveals adaptive innovations driving the diversification of true bugs. Proceedings of the Royal Society B: Biological Sciences 284(1862): e20171223. https://doi.org/10.1098/rspb.2017.1223

Li HM, Deng RQ, Wang JW, Chen ZY, Jia FL, Wang XZ (2005) A preliminary phylogeny of the Pentatomomorpha (Hemiptera: Heteroptera) based on nuclear 18S rDNA and mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 37(2): 313–326. https://doi.org/10.1016/j.ympev.2005.07.013

Li R, Li M, Yan J, Bai M, Zhang H (2021) Five Mitochondrial Genomes of the Genus *Eysarcoris* Hahn, 1834 with Phylogenetic Implications for the Pentatominae (Hemiptera: Pentatomidae). Insects 12(7): 597. https://doi.org/10.3390/insects12070597

Li Y, Kocot KM, Schander C, Santos SR, Thornhill DJ, Halanych KM (2015) Mitogenomics reveals phylogeny and repeated motifs in control regions of the deep-sea family Siboglinidae (Annelida). Molecular Phylogenetics and Evolution 85: 221–229. https://doi.org/10.1016/j.ympev.2015.02.008

Liu Y, Li H, Song F, Zhao Y, Wilson JJ, Cai W (2019) Higher-level phylogeny and evolutionary history of Pentatomomorpha (Hemiptera: Heteroptera) inferred from mitochondrial genome sequences. Systematic Entomology 44(4): 810–819. https://doi.org/10.1111/syen.12357

Liu Q, Zheng LY, Nonnajzab (1994) The classification and faunal analyses of Rhopalidae (Hemiptera) in China. Journal of Arid Land Resources and Environment 8(3): 102–115.

Li XZ (1996) Cladistic analysis and higher classification of Coreoidea (Heteroptera). Entomologia Sinica 3(4): 283–292. https://doi.org/10.1111/j.1744-7917.1996.tb00277.x

Nonnaizab (1986) Fauna of Inner Mongolia (Hemiptera: Heteroptera). Inner Mongolia People's Publishing House, Hohhot, 298 pp.

Ojala D, Montoya J, Attardi G (1981) tRNA punctuation model of RNA processing in human mitochondria. Nature 290(5806): 470–474. https://doi.org/10.1038/290470a0

Quek ZBR, Chang JJM, Ip YCA, Chan YKS, Huang D (2021) Mitogenomes reveal alternative initiation codons and lineage-specific gene order conservation in echinoderms. Molecular Biology and Evolution 38(3): 981–985. https://doi.org/10.1093/molbev/msaa262

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): 901. https://doi.org/10.1093/sysbio/syy032

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic infer-

ence and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Schaefer CW (1993) Origins of the New World Rhopalinae (Hemiptera: Rhopalidae). Annals of the Entomological Society of America 86(2): 127–133. https://doi.org/10.1093/aesa/86.2.127

Schaefer CW, Chopra NP (1982) Cladistic analysis of the Rhopalidae, with a list of food plants. Annals of the Entomological Society of America 75(3): 224–233. https://doi.org/10.1093/aesa/75.3.224

Schaefer CW, Panizzi AR (2000) Scentless plant bugs (Rhopalidae). In: Schaefer CW, Panizzi AR (Eds) Heteroptera of Economic Importance. CRC Press, Boca Raton, 331–342. https://doi.org/10.1201/9781420041859

Simon C, Buckley TR, Frati F, Stewart JB, Beckenbach AT (2006) Incorporating molecular evolution into phylogenetic analysis and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. Annual Review of Ecology, Evolution and Systematics 37: 545–579. https://doi.org/10.1146/annurev.ecolsys.37.091305.110018

Song N, Zhang H, Zhao T (2019) Insights into the phylogeny of Hemiptera from increased mitogenomic taxon sampling. Molecular phylogenetics and evolution 137: 236–249. https://doi.org/10.1016/j.ympev.2019.05.009

Song N, Liang AP, Bu CP (2012) A molecular phylogeny of Hemiptera inferred from mitochondrial genome sequences. PloS ONE 7(11): e48778. https://doi.org/10.1371/journal.pone.0048778

Souza HV, Souza FB, Maruyama SRC, Castanhole MMU, Itoyama MM (2009) Meiosis, spermatogenesis and nucleolar behavior in the seminiferous tubules of Alydidae, Coreidae and Rhopalidae (Heteroptera) species. Genetics and Molecular Research 8(4): 1383–1396 https://doi.org/10.4238/vol8-4gmr672

Souza-Firmino TSD, Alevi KCC, Itoyama MM (2020) Chromosomal divergence and evolutionary inferences in Pentatomomorpha infraorder (Hemiptera, Heteroptera) based on the chromosomal location of ribosomal genes. Plos ONE 15(2): e0228631. https://doi.org/10.1590/S0074-02762013000300017

Stamatakis A (2015) Using RAxML to infer phylogenies. Current Protocols in Bioinformatics 51(1): 6–14. https://doi.org/10.1002/0471250953.bi0614s51

Steill J, Meyer J (2003) The Rhopalidae of Florida. Insect Classification Project 4 (30): 1–23. http://entnemdept.ufl.edu/choate/rhopalidae.pdf

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Tian XX, Xie Q, Li M, Gao CQ, Cui Y, Xi L, Bu W (2011) Phylogeny of pentatomomorphan bugs (Hemiptera-Heteroptera: Pentatomomorpha) based on six Hox gene fragments. Zootaxa 2888: 57–68. https://doi.org/10.11646/zootaxa.2888.1.5

Tyagi K, Chakraborty R, Cameron SL, Sweet AD, Chandra K, Kumar V (2020) Rearrangement and evolution of mitochondrial genomes in Thysanoptera (Insecta). Scientific reports 10(1): 1–16. https://doi.org/10.1038/s41598-020-57705-4

Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27(2): 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Wolstenholme DR (1992) Animal mitochondrial DNA: structure and evolution. International Review of Cytology 141: 173–216. https://doi.org/10.1016/S0074-7696(08)62066-5

Weirauch C, Schuh RT (2011) Systematics and evolution of Heteroptera: 25 years of progress. Annual Review of Entomology 56: 487–510. https://doi.org/10.1146/annurev-ento-120709-144833

Xu N, Ding J, Que Z, Xu W, Ye W, Liu H (2021) The mitochondrial genome and phylogenetic characteristics of the Thick-billed Green-Pigeon, *Treron curvirostra*: the first sequence for the genus. ZooKeys 1041: 167–182. https://doi.org/10.3897/zookeys.1041.60150

Xie Q, Bu W, Zheng L (2005) The Bayesian phylogenetic analysis of the 18S rRNA sequences from the main lineages of Trichophora (Insecta: Heteroptera: Pentatomomorpha). Molecular Phylogenetics and Evolution 34: 448–451. https://doi.org/10.1016/j.ympev.2004.10.015

Yang QQ, Liu SW, Song F, Liu GF, Yu XP (2018) Comparative mitogenome analysis on species of four apple snails (Ampullariidae: Pomacea). International Journal of Biological Macromolecules 118: 525–533. https://doi.org/10.1016/j.ijbiomac.2018.06.092

Yuan ML, Zhang QL, Guo ZL, Wang J, Shen YY (2015) The complete mitochondrial genome of Corizus tetraspilus (Hemiptera: Rhopalidae) and phylogenetic analysis of Pentatomomorpha. PLoS ONE 10(6): e0129003. https://doi.org/10.1371/journal.pone.0129003

Zhao L, Wei J, Zhao W, Chen C, Gao X, Zhao Q (2021) The complete mitochondrial genome of *Pentatoma rufipes* (Hemiptera, Pentatomidae) and its phylogenetic implications. ZooKeys 1042: 51–72. https://doi.org/10.3897/zookeys.1042.62302

Zhao W, Zhao Q, Li M, Wei J, Zhang X, Zhang H (2019) Comparative mitogenomic analysis of the *Eurydema* genus in the context of representative Pentatomidae (Hemiptera: Heteroptera) taxa. Journal of Insect Science 19(6): 20. https://doi.org/10.1093/jisesa/iez122

Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Research 31(13): 3406–3415. https://doi.org/10.1093/nar/gkg595